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Effects of raubasine stereoisomers on pre- and postsynaptic α-adrenoceptors in the rat vas deferens

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1 The actions of raubasine, tetrahydroalstonine and akuammigine were studied on pre- and postsynaptic α-adrenoceptors of the rat vas deferens.

2 These three drugs competitively antagonized the effect of noradrenaline on postsynaptic α-adrenoceptors, yielding pA_2 values of 6.57, 4.56 and 4.68 respectively.

3 The presynaptic α-adrenoceptor antagonist activity of the drugs was quantitatively determined by studying the effect of increasing concentrations on the clonidine dose-response curve in the electrically stimulated vas deferens.

4 The inhibitory effect of clonidine could be competitively blocked by these three compounds and the pA_2 values for raubasine, tetrahydroalstonine and akuammigine were 6.02, 7.71 and 5.64 respectively.

5 These results indicate that: akuammigine is a very weak antagonist at pre- and postsynaptic sites; raubasine acts preferentially at postsynaptic sites; tetrahydroalstonine is a highly selective presynaptic α-adrenoceptor blocking agent. The ratio of the pre/postsynaptic potency declines in the order tetrahydroalstonine > akuammigine > raubasine.

Introduction

Studies on pre- and postsynaptic α-adrenoceptors of sympathetic neuroeffector junctions have generated the belief that α-adrenoceptors can be divided into α_1- and α_2-subtypes. These two types of α-adrenoceptor differ in their sensitivities to agonists and antagonists (Starke, 1981; McGrath, 1982).

Raubasine is a 3α - 5α - 20β - 16,17 didehydro - 19α - methyl - oxayohimban - 16 carboxylic acid methylester and is one isomeric form of the structure shown in Figure 1. This structure contains 4 asymmetric carbon atoms (3, 15, 19, 20) and should therefore exist in 16 isomeric forms. Eight of these isomers have been isolated from natural material (Melchio *et al.*, 1977) and are listed in Table 1. Three asymmetric carbons (3, 15, 20) occupy positions at ring junctions and so alteration of their stereochemical conformations should lead to marked changes in the shape of the molecules.

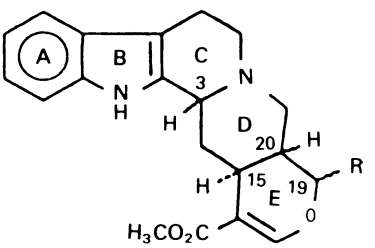

Figure 1 Generalized ring structure of raubasine, $R = CH_3$. Orientation of R and/or ring-junction hydrogens are normally indicated with an α for downward projecting groups and a β for upward projecting groups.

Table 1 The 8 known raubasine isomers

Isomer	*Stereochemistry*			
	H-3	H-15	H-19	H-20
Rauniticine	α	α	α	α
Iso-3-rauniticine	β	α	α	α
Raubasine	α	α	β	β
Iso-3-ajmalicine	β	α	β	β
Epi-19-ajmalicine	α	α	α	β
Iso-3-epi 19-ajmalicine	β	α	α	β
Tetrahydroalstonine	α	α	β	α
Akuammigine	β	α	β	α

Stereochemical data from Melchio *et al.* (1977).

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The adrenolytic and sympatholytic properties of raubasine have been described by many authors (Achelis & Kroneberg, 1953; Kroneberg & Achelis, 1954; Schmitt & Gonnard, 1957; Kroneberg, 1958; Raymond-Hamet & Rothlin, 1960; Roquebert *et al.*, 1981). In a previous publication (Demichel *et al.*, 1981) we have shown that in the rat vas deferens, raubasine preferentially blocks post-synaptic α-adrenoceptors. The purpose of the present study was to compare the relative potencies of its two isomers, tetrahydroalstonine and akuammigine to raubasine itself, at pre- and postsynaptic α-adrenoceptors in the rat vas deferens, where only one population of post-junctional α-adrenoceptor, the α_1-type is present (Docherty *et al.*, 1979; Doggrell, 1981).

Methods

Rat isolated vas deferens

Male Wistar rats (230 ± 20 g) were killed by a blow on the head and exsanguinated. The vasa deferentia were removed and each vas was suspended under a preload of 0.5 g in a 20 ml muscle chamber containing Krebs solution at 37°C and gassed with 95% O_2:5% CO_2. The composition of the Krebs solution was (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1. After a 30 min stabilization period, contractions were measured with an isotonic myograph transducer and a strip chart recorder.

Field stimulation

A straight platinum electrode (10 mm in length) was placed in contact with the lower end, and a circular platinum electrode (5 mm in diameter) was placed round the upper end of the vas deferens. The intramural nerves were continuously stimulated by rectangular pulses: 2 ms duration, 30 V, 0.1 Hz which normally give the maximal response. This stimulation condition remained constant throughout the experiment.

Pre- and postsynaptic activity

The pre- and postsynaptic α-adrenoceptor blocking activity of the drugs was assessed as previously described in detail by Demichel *et al.* (1981).

The blocking activity of the α-adrenoceptor antagonists on postsynaptic α-adrenoceptors was evaluated by their antagonism of (−)-noradrenaline-induced contraction of the vas deferens. Noradrenaline was added to the bath in increasing concentrations, so that a cumulative concentration-response curve was produced. After two successive concentration-response curves of equal size had been obtained, a given quantity of antagonist (one dose per tissue) was introduced into the chamber 5 min before a new cumulative curve was established for the agonist. Results are expressed as percentage of maximally induced contraction. Blocking activity of the α-antagonists on the presynaptic α-adrenoceptors was evaluated by their antagonism of the effects of α_2-adrenoceptor agonist, clonidine, which inhibits twitch responses of the field stimulated vas deferens, by acting on the presynaptic α-adrenoceptors. The twitches being constant, increasing concentrations of clonidine were added to the bath every 3 min without washing. The inhibitory effect of each concentration on the twitch response was expressed as a percentage inhibition of the maximal contraction. Thus, it was

Table 2 Drug antagonism at pre- and postsynaptic α-adrenoceptors of the rat vas deferens

	Drug parameter			
	Presynaptic		*Postsynaptic*	
Antagonist	pA_2	Slope	pA_2	Slope
Raubasine	6.02 ± 0.07	1.02 ± 0.06	6.57 ± 0.04	0.96 ± 0.02
Tetrahydro-alstonine	7.71 ± 0.23	1.25 ± 0.08	4.56 ± 0.05	1.07 ± 0.06
Akuammigine	5.64 ± 0.08	1.1 ± 0.15	4.68 ± 0.05	0.91 ± 0.04
Agonist	pD_2		pD_2	
Clonidine	8.50 ± 0.12			
Noradrenaline			5.35 ± 0.15	

pA_2 values ± s.e.mean and slopes of regression lines were calculated from Schild plots (Arunlakshana & Schild, 1959); pD_2 values ± s.e.mean were determined according to Van Rossum (1963).

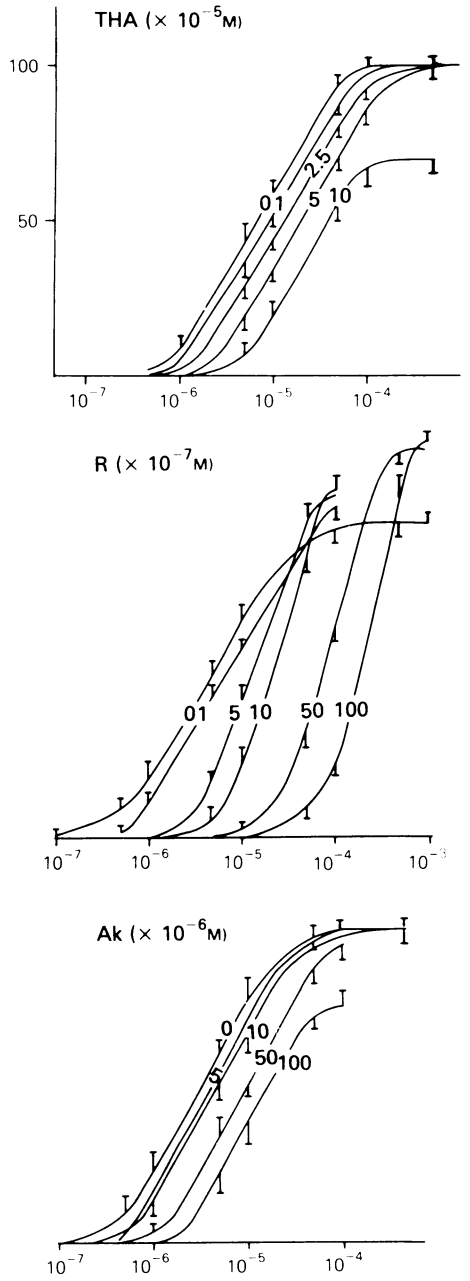

Figure 2 Cumulative concentration-response curve to noradrenaline in the rat vas deferens in the presence of tetrahydroalstonine (THA), raubasine (R), akuammigine (Ak). Ordinate scale: % of maximal noradrenaline response. Abscissa scale: – log molar concentration of noradrenaline. Each point represents the mean of n = 20–25 for control and 5 for each antagonist concentration; vertical lines show s.e.mean.

possible to construct cumulative concentration-response curves for clonidine. After producing two successive and comparable control curves (clonidine alone) the sensitivity of the preparation towards clonidine was again determined after 10 min in contact with an antagonist. In both case, the antagonist drugs remained in the bathing fluid for the duration of the concentration-response curve determination. Five isolated organs were used for each antagonist concentration.

Expression of results

The pA_2 values, parameters of conventional affinity in the case of competitive antagonism were determined as described by Arunlakshana & Schild (1959). The affinity of agonists is expressed by their pD_2 values, determined according to the method of Van Rossum (1963).

Drugs

(–)-Noradrenaline bitartrate (Koch-Light) and clonidine hydrochloride (Boehringer Ingelheim) were dissolved in Krebs solution. Raubasine, tetrahydroalstonine and akuammigine (P. Fabre Laboratories) were dissolved in 1% tartaric acid (35 mg drug, 10 ml tartaric acid solution) and further diluted with Krebs solution.

Statistical analysis

Statistical analysis was performed with Student's *t* test.

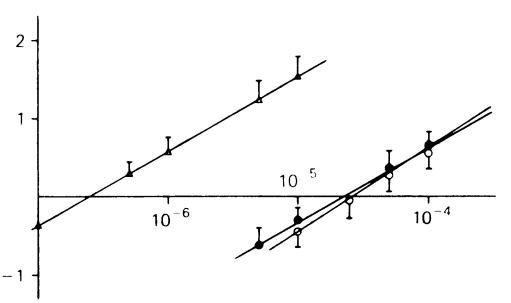

Figure 3 Schild plot of the results shown in Figure 2, for the tetrahydroalstonine (○), raubasine (△) and akuammigine (●), antagonism of noradrenaline-induced contractions of the rat isolated vas deferens. Ordinate scale: log (x – 1) where x = ratio of the agonist concentration required to obtain the same fraction of the maximal effect in the presence or absence of antagonist. Abscissa scale: – log molar concentration of antagonist. Each point is the mean of 5 experiments; vertical lines show s.e.mean.

Results

Antagonist activity at postsynaptic α-adrenoceptors

Raubasine (10^{-7} to 10^{-5} M), tetrahydroalstonine (10^{-5} to 10^{-4} M) and akuammigine (5×10^{-6} to 10^{-4} M) caused a parallel displacement to the right of the concentration-response curves to noradrenaline (Figure 2), without depressing the maximal contraction, except for akuammigine at 10^{-4} M. These results suggest competitive antagonism. The Schild plots (Arunlakshana & Schild, 1959) gave a linear regression to the three drugs, and the pA_2 values determined from zero intercept (Figure 3) are shown in Table 2. The effects of these three substances were easily reversed by adding fresh perfusion fluid. The decreasing order of affinity for the postsynaptic α-receptors was: raubasine > akuammigine > tetrahydroalstonine. The pA_2 value for raubasine was significantly greater ($P < 0.01$) than the values obtained for tetrahydroalstonine and akuammigine. There was no statistically significant difference between the values for tetrahydroalstonine and akuammigine.

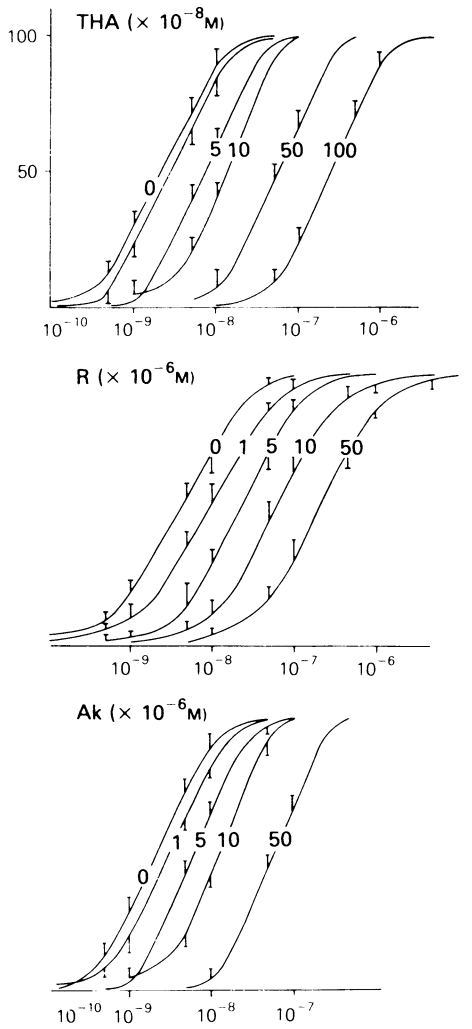

Figure 4 Antagonism of the twitch-inhibitory effect of clonidine by tetrahydroalstonine (THA), raubasine (R), akuammigine (Ak), in electrically stimulated rat vas deferens (0.1 Hz, 30 V, 2 ms). Ordinate scale: % of maximal inhibition. Abscissa scale: − log molar concentration of clonidine. Each point is the mean of $n = 20$–25 for control and 5 for each antagonist concentration; vertical lines show s.e.mean.

Antagonist activity at presynaptic α-adrenoceptors

The contraction induced by electrical stimulation of the rat isolated vas deferens results from postganglionic intramural nerve stimulation. Clonidine caused a concentration-dependent inhibition of the twitch responses, by a mechanism involving presynaptic α-adrenoceptors (Demichel *et al.*, 1981). Raubasine (10^{-5} to 5×10^{-5} M) and tetrahydroalstonine (10^{-4} M) enhanced the twitch responses to nerve stimulation, but akuammigine (10^{-5} to 5×10^{-5} M) had no effect. To eliminate the influence of the antagonists themselves on the twitch response, each set of responses was expressed as a percentage of its own maximum.

Raubasine (5×10^{-7} to 5×10^{-5} M), tetrahydroalstonine (10^{-8} to 10^{-6} M) and akuammigine (10^{-6} to

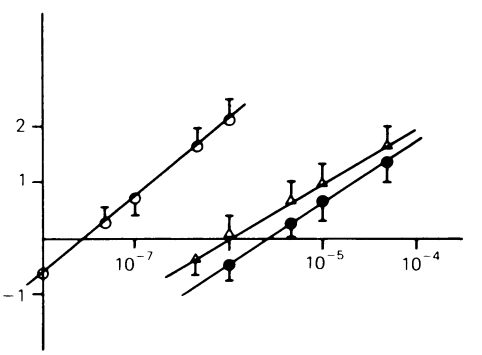

Figure 5 Schild plot of the results shown in Figure 4 for the tetrahydroalstonine (○), raubasine (△), akuammigine (●), antagonism of clonidine-induced inhibition of contraction in electrically stimulated rat vas deferens. Ordinate scale: log (x − 1) where x = ratio of the agonist concentration required to obtain the same fraction of the maximal effect in the presence or absence of antagonist. Abscissa scale: − log molar concentration of antagonist. Each point is the mean of 5 experiments; vertical lines show s.e.mean.

Table 3 Antagonist potency at pre- and postsynaptic α-adrenoceptors

Compound	*Postsynaptic molar concentration*	*Presynaptic molar concentration*	*Ratio pre/post*
Raubasine	2.69×10^{-7}	9.54×10^{-7}	0.281
Tetrahydroalstonine	2.75×10^{-5}	1.95×10^{-8}	1410
Akuammigine	2.08×10^{-5}	2.29×10^{-6}	9

pA_2 values were calculated from Schild plots and then converted to their respective molar concentration. The pre/postsynaptic ratios were calculated from these concentrations.

5×10^{-5} M) caused parallel concentration-dependent shifts of the clonidine concentration-response curves to the right (Figure 4) without reduction of the maximum inhibitory response, suggesting a competitive type of antagonism. Schild plots (Arunlakshana & Schild, 1959) gave linear regressions in each case (Figure 5). The pA_2 values and slopes of the regression lines are given in Table 2. The decreasing order of affinity was: tetrahydroalstonine > raubasine > akuammigine. The pA_2 values for tetrahydroalstonine were significantly greater ($P < 0.01$) than those for raubasine and akuammigine.

Discussion

The three drugs blocked postsynaptic α-adrenoceptors in the noradrenaline-stimulated vas deferens. The slope of the Schild plots for all the antagonists tested was close to unity (Table 2) indicating competitive antagonism. Differences in affinity for the postsynaptic α-receptors was shown by the different pA_2 values. Raubasine had much greater affinity than either tetrahydroalstonine or akuammigine, which both had very weak activity.

In the experiments on the intramurally stimulated rat vas deferens, clonidine produced a concentration-dependent inhibition of the twitch response which was competitively antagonized by the three drugs. The slopes of the regression lines were not significantly different from unity (Table 2). There were distinct differences in affinity for the presynaptic α-receptors as shown by the pA_2 values. Raubasine and akuammigine had much lower affinities than tetrahydroalstonine.

A selective effect of each α-antagonist on either pre- or postsynaptic α-adrenoceptors was assessed by the ratio of their molar concentrations calculated from pA_2 values towards noradrenaline and clonidine respectively (Table 3). The decreasing order of selectivity for presynaptic α-adrenoceptors was: tetrahydroalstonine > akuammigine > raubasine.

This study showed that tetrahydroalstonine was about 1.400 times more active in blocking the inhibitory action of clonidine on the twitch response, than in blocking noradrenaline-induced contractions of the rat vas deferens. Since the action of clonidine is mediated by presynaptic α_2-adrenoceptors (Starke *et al.*, 1974) which are quite different from the postsynaptic α_1-type, tetrahydroalstonine can be considered to have considerable selectivity towards presynaptic α_2-adrenoceptors. It is greater than that of yohimbine (pre/post ratio for yohimbine is 112, Demichel *et al.*, 1981). Raubasine on the other hand, can be considered as a drug that preferentially blocks postsynaptic α-adrenoceptors (significant difference in pA_2 to clonidine and noradrenaline: $P < 0.01$). Akuammigine which acts predominantly at presynaptic α-adrenoceptors is a very weak antagonist at both pre- and postsynaptic α-adrenoceptors.

The different chemical structures of the three drugs can be invoked to explain the differential pre-/postsynaptic activities of these three drugs. Akuammigine which has a 3β-configuration, is twisted about the C/D ring junction (Melchio *et al.*, 1977) and had very weak α-adrenolytic activity. Raubasine and tetrahydroalstonine have the 3α-configuration (similar to yohimbine and corynanthine; Morrison, 1967), and are characterized by a relatively planar ring structure. These two drugs differ in their pre- and postsynaptic potencies, raubasine is more active at post- than at presynaptic receptors. Tetrahydroalstonine is 50 times more active at presynaptic sites, and 100 times less active than raubasine at postsynaptic sites.

Raubasine and tetrahydroalstonine differ in their stereochemistry at C_{20}, which is β in raubasine and α in tetrahydroalstonine. In raubasine the D/E ring junction is '*trans*' while in tetrahydroalstonine it is '*cis*'. The *cis* configuration appears to increase considerably selectivity towards presynaptic α_2-adrenoceptors. This would suggest that a flat upper surface to the molecule is required for high α-adrenoceptor affinity, and that the stereochemistry

of C_{20} is important in the differing selectivities for pre- and postsynaptic α-adrenoceptors of this series of compounds.

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